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Optimization of cholinesterase-based catalytic bioscavengers against organophosphorus agents

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Abstract

© 2018 Lushchekina, Schopfer, Grigorenko, Nemukhin, Varfolomeev, Lockridge and Masson. Organophosphorus agents (OPs) are irreversible inhibitors of acetylcholinesterase (AChE). OP poisoning causes major cholinergic syndrome. Current medical counter-measures mitigate the acute effects but have limited action against OP-induced brain damage. Bioscavengers are appealing alternative therapeutic approach because they neutralize OPs in bloodstream before they reach physiological targets. First generation bioscavengers are stoichiometric bioscavengers. However, stoichiometric neutralization requires administration of huge doses of enzyme. Second generation bioscavengers are catalytic bioscavengers capable of detoxifying OPs with a turnover. High bimolecular rate constants (kcat/Km> 106M-1min-1) are required, so that low enzyme doses can be administered. Cholinesterases (ChE) are attractive candidates because OPs are hemi-substrates. Moderate OP hydrolase (OPase) activity has been observed for certain natural ChEs and for G117H-based human BChE mutants made by site-directed mutagenesis. However, before mutated ChEs can become operational catalytic bioscavengers their dephosphylation rate constant must be increased by several orders of magnitude. New strategies for converting ChEs into fast OPase are based either on combinational approaches or on computer redesign of enzyme. The keystone for rational conversion of ChEs into OPases is to understand the reaction mechanisms with OPs. In the present work we propose that efficient OP hydrolysis can be achieved by re-designing the configuration of enzyme active center residues and by creating specific routes for attack of water molecules and proton transfer. Four directions for nucleophilic attack of water on phosphorus atom were defined. Changes must lead to a novel enzyme, wherein OP hydrolysis wins over competing aging reactions. Kinetic, crystallographic, and computational data have been accumulated that describe mechanisms of reactions involving ChEs. From these studies, it appears that introducing new groups that create a stable H-bonded network susceptible to activate and orient water molecule, stabilize transition states (TS), and intermediates may determine whether dephosphylation is favored over aging. Mutations on key residues (L286, F329, F398) were considered. QM/MM calculations suggest that mutation L286H combined to other mutations favors water attack from apical position. However, the aging reaction is competing. Axial direction of water attack is not favorable to aging. QM/MM calculation shows that F329H+F398H-based multiple mutants display favorable energy barrier for fast reactivation without aging.

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Keywords

Acetylcholinesterase, Bioscavenger, Butyrylcholinesterase, Computer design,

Organophosphorus compound, Phosphotriesterase

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